Lowe CR, Burton SJ, Burton NP *et al.* (1992) Designer dyes: 'biomimetic' ligands for the purification of pharmaceutical proteins by affinity chromatography. *TIBTECH* 10: 442.

Palanisamy UD, Hussain A, Iqbal S *et al.* (1999) Design, synthesis and characterisation of affinity ligands for glycoproteins. *Journal of Molecular Recognition* 12: 57.

Smith GP (1985) Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virion surface. *Science* 228: 1315.

Starkenstein EV (1910) Über fermentenwir-kungund deren beeinflussung durch neutralsalze. *Biochemische Zeitschrift* 24: 14.

Teng SF, Sproule K, Hussain A and Lowe CR (1999) A strategy for the generation of biomimetic ligands for affinity chromatography. Combinatorial; synthesis and biological evaluation of an IgG binding ligand. *Journal of Molecular Recognition* 12: 67.

Tuerk C and Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 249: 505.

# Theory and Development of Affinity Chromatography

**R. Scopes**, La Trobe University, Bundoora, Melbourne, Australia

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## Introduction

The term affinity chromatography began to be used extensively in the 1960s to describe protein separation methods that made use of the specific biological interaction of the desired protein with some ligand that was immobilized on an adsorbent matrix. Since most proteins, and all enzymes, bind to some compound very specifically, this immediately promised to solve most protein purification problems. But, as with all good ideas, there were many cases when it did not work as expected; the general concept of affinity chromatography for purifying proteins found its niche, but was no panacea. More recently, it has found a fairly widespread application in purifying recombinant proteins, using very standardized procedures.

Although most applications have been for proteins, it is not so limited in theory, since other biological macromolecules have specific interactions which can be exploited, especially nucleic acids. But, for the purposes of this article, the principles will be expounded with proteins as the prime target. We should consider the definition(s) of affinity chromatography carefully, since it does not mean the same to everyone. First, the word affinity. Any two components that are attracted to each other can be said to have an affinity, but if we took that as a definition, the term would be too broad to be useful – for instance, it could include all types of chromatography. It is better to limit the definition of affinity to a biologically significant interaction such as between a hormone and its receptor, an enzyme and its substrate, or an antibody and its antigen. Unfortunately, there are well-established uses of the term, such as immobilized metal affinity chromatography, in which the interaction is not biologically relevant, though it too can be highly specific. Perhaps a better definition could imply simply a high specificity and selectivity of the interaction, though that can exclude some examples of true biological affinity.

The other word, chromatography, strictly means that process in which components are adsorbed and desorbed continuously as they move down a column, or through some other medium, resulting in a multistage separation of different components according to their partitioning between the stationary and mobile phases. But affinity methods are usually treated in an 'on–off' fashion in which, after total adsorption of the desired component, a stepwise change in the buffer mobile phase results in its complete elution, and true chromatography is not carried out. Nevertheless, the word chromatography is used more widely than its strict definition, to encompass any use of an adsorbent, even in this 'on–off' fashion.

And so we come up with a definition of affinity chromatrography as a chromatographic procedure utilizing an adsorbent involving an immobilized ligand which has a high specificity for binding the desired component, preferably to the exclusion of all others. This binding can be loosened by a change in buffer conditions, to elute the desired component relatively free of contaminants. If the ligand is the natural biological ligand of the desired component, then the more precise term bioaffinity chromatography can be used. On the other hand, when the interaction is specific, but the ligand is unnatural, terms such as pseudo-affinity chromatography and biomimetic chromatography have been adopted.

## Early Developments in Affinity Chromatography

The first protein to by purified by affinity chromatography was amylase in 1910, for which a column

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packed with starch was used. Presumably the column disintegrated during the process! Attempts were made in the 1950s to link antigens to cellulose columns for the purification of antibodies, but these were not very successful because of the low capacity of the cellulose matrix. Unless the particles used to pack the column are permeable to the proteins, only the surface of the particles is available for attachment. The more successful applications commenced when suitable protein-permeable particulate materials were developed, together with reliable chemical methods that could be used for attaching specific ligands to these materials. Much of the original work was based on the use of cyanogen bromide as a method for activating the matrix, and the use of agarose beads as the support material. Agarose has almost ideal properties, in that the gel formation of the beads has pores permeable to most proteins, so that the internal volume of the beads is all available for attachment, as well as the outside surface. Moreover, agarose in itself has virtually no affinity for any protein (other than agarases), so nonspecific binding is rarely observed. It is still the matrix of choice for most affinity chromatography, though there are now many competing materials, both polysaccharide-based and synthetic.

Activation of the matrix involves treating the material with a chemical that introduces a group (normally reacting with hydroxyls on polysaccharide matrices) which itself will then react with something, usually an amine, in the ligand to be used. All such activating chemicals are extremely reactive and dangerous to handle, especially those that are volatile. Cyanogen bromide reacts to introduce several forms of cyanate derivatives, of which the cyanate ester is the most important. This ester will couple to a primary amine, such as a lysine residue in a protein, to produce the isourea-linked ligand (**Figure 1**).

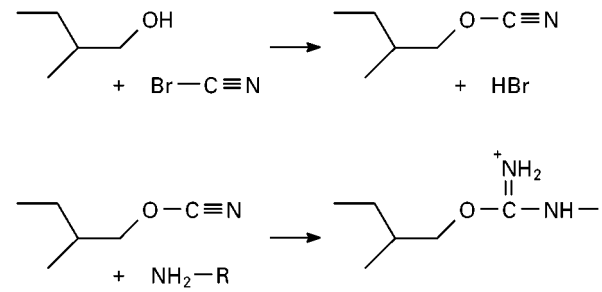


**Figure 1** Activation of a carbohydrate matrix with cyanogen bromide. The major product is the cyanate ester after reaction with a primary hydroxyl group. This rapidly couples with an amine to produce the isourea structure, which is positively charged at neutral pH.

Cyanogen bromide activation is still widely used, and the dangerous chemistry is overcome by having the already-activated agarose available as a commercial product. However, it does have some disadvantages compared with other methods outlined below – in particular, the instability of the isourea linkage.

Many other activation methods have been developed, and a brief summary of these is given in **Table 1**. Several of these incorporate a bifunctional reagent, one end of which combines with the matrix, and the other with the ligand. These may cause some cross-linking within the matrix, but this can be advantageous for matrix stability. Bifunctional reagents also introduce a spacer arm (see below) of various lengths depending on the reagent. The activated matrix then reacts with a nucleophile such as an amine, or in some cases a sulfhydryl group in the ligand being coupled (**Figure 2**A).

The ligands exploited at first were mainly enzyme substrates. In particular, so-called group-specific ligands were developed which could be used for a variety of different enzymes having the same

**Table 1** A selection of activation methods, and the properties of the spacer arms introduced after coupling with amino-reactive ligand. A positive charge can result in some nonspecific anion exchange behaviour at low ionic strengths. Cleaning of protein adsorbents is best carried out with alkali, but many linkages are alkali-labile

| *Reagent* | *Spacer arm length, atoms* | *Type of linkage* | *Charge at pH 7* | *Alkali lability* |
|---|---|---|---|---|
| Cyanogen bromide | 1 | Isourea | + | Yes |
| Carbodiimide | 1 | Amide | 0 | Yes |
| Epichlorhydrin | 3 | Secondary amine | + | No |
| Bisoxirane | 11 | Secondary amine | + | No |
| Divinyl sulfone | 5 | Secondary amine | + | Yes |
| Tosyl/tresyl | 0 | Secondary amine | + | No |
| Hydroxy-succinimide | 8[a] | Amide | 0 | Yes |
| Cyanuric chloride | 4 | Aromatic amine | 0 | No |

[a]Depends on activation reagents.

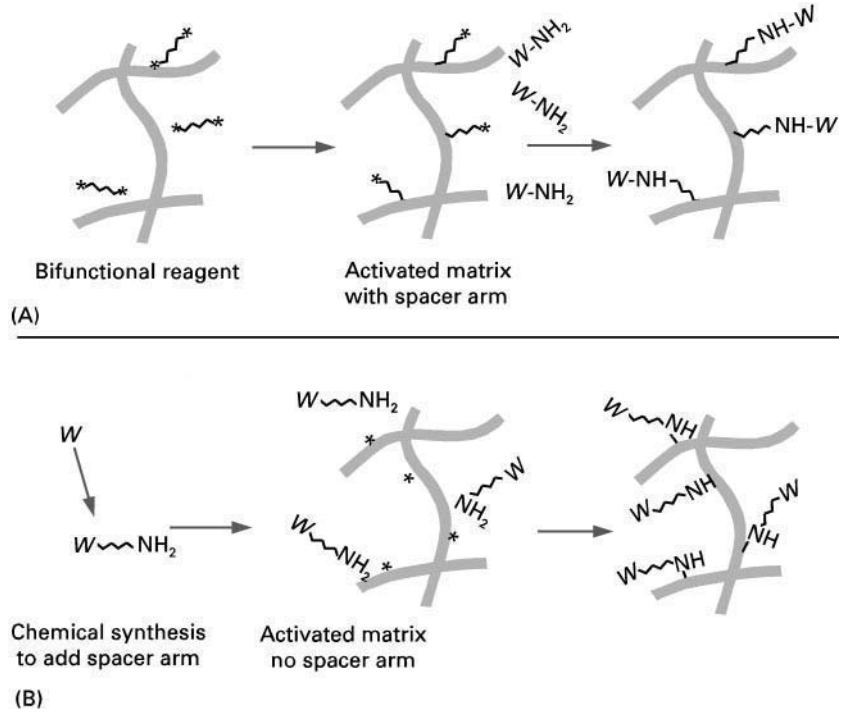


**Figure 2** (A) Activation of a matrix with a bifunctional reagent, which provides a spacer arm. (B) Coupling of a ligand containing a built-in spacer arm to an activated matrix.

substrate – more particularly, a common cofactor. Thus we had, and can still purchase, adsorbents containing nucleotide cofactors such as ATP and NAD, lectins for glycoproteins and nucleic acids for binding either other nucleic acids or enzymes involved in nucleic acid metabolism. The use of affinity chromatography which exploits the interaction between an antibody and its antigen has been extensively developed, and is described in detail elsewhere.

The main problems associated with affinity chromatography soon appeared. These can be summarized as:

1. It took a long time to develop an adsorbent that does the job.
2. Once made, the adsorbent is expensive and has a limited useful life.
3. There is nonspecific binding of unwanted proteins.
4. There is a need for spacer arms.
5. There are difficulties in satisfactory elution.

Many of these problems have now been solved, with a clearer understanding of the important factors involved. It is now not often that a completely new adsorbent has to be developed. The expense parameter is less important on a research scale, but is a major consideration in large scale commercial purification. The related problems of nonspecific binding and spacer arms can usually be overcome by judicious process design, and elution procedures for the more difficult tasks such as antibody–antigen interactions are now better established. Even so, there are many cases in which a workable true affinity method cannot be established, usually because the natural affinity may be very specific, but quantitatively weak.

## Spacer Arms

The need for spacer arms was realized early on, since placing the ligand directly adjacent to the matrix might sterically hinder the interaction with the protein (**Figure 3**). But it has been shown that, as most spacer arms are hydrophobic, they can interact with other parts of the protein in a relatively nonspecific way. This has both advantages and disadvantages: the advantage, as described below, is that these nonspecific hydrophobic interactions can add to the binding strength of otherwise weak, though highly specific, interactions between a protein and its natural ligand. When inert, i.e. hydrophilic, spacer arms were introduced, many previously successful affinity methods did not work because the binding of the desired component was now too weak. The disadvantage of the use of spacer arms is that hydrophobic interactions with unwanted proteins may be so strong that these proteins may be bound as well.

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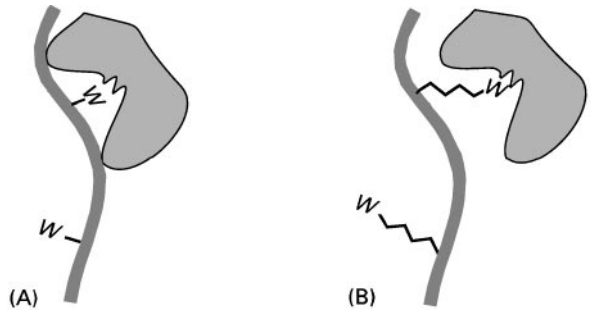


**Figure 3** Demonstration of the role of a spacer arm in keeping the bound protein away from the matrix backbone. Without the arm, it may be impossible for the protein to interact with the ligand, *W*.

Spacer arms can either be introduced as part of the activation process (see **Table 1**), or added afterwards in a multi-step process of creating the adsorbent. Sometimes it is beneficial to synthesize chemically a suitable spacer arm (ending with an amine) attached to the ligand itself, when the ligand does not have a suitable active nucleophile for direct coupling (Figure 2B).

Thus, spacer arms have two possible functions in a successful affinity adsorbent. First, they place the ligand away from the matrix to avoid physical interference from the matrix backbone. Second, they can provide some weak nonspecific interactions that increase the overall binding energy so that adsorption is more complete.

## Some Quantitative Parameters

For a protein to bind to an adsorbent in a column, it must have sufficient affinity (in the broadest sense) to partition to the solid phase. It need not be an absolute adsorption; the protein can be in a dynamic state between being adsorbed and in solution. An equilibrium can be established provided that the flow rate is not too great, and an equilibrium constant defined between the matrix binding sites and the protein itself. We can call this the affinity constant, equal to the association constant between the protein and the adsorbent. If the protein is to bind to the adsorbent, it must spend most of its time ($> 90\%$) in the adsorbed state so that its progress down the column is greatly delayed. We can calculate that, for strong binding the affinity constant will typically need to be at least in the range $10^{-5}$–$10^{6}$ mol $L^{-1}$. Alternatively, the reciprocal of this value may be expressed as a dissociation constants – 10 μmol $L^{-1}$. It is useful to use dissociation constants, since they can be related to an enzyme's Michaelis constant value, which is easily determined from the enzyme's activity at different substrate concentrations. The value of the required affinity constant depends very much on the effective concentration of binding sites in the matrix. Although the concentration of ligand attached to the matrix may be quite high, for steric reasons it is usually the case that only a small percentage are actually available for binding.

Since many enzymes do not bind their substrates with micromolar affinities, it appears that true affinity chromatography is often not possible. Nevertheless, it was soon discovered that strong binding could be obtained despite relatively high dissociation constants (i.e. weak binding) for the free ligand and the protein, and successful procedures could be developed. This was because nonspecific binding, mainly due to spacer arm interactions, increased the overall adsorption.

If we define a binding free energy for the biospecific interaction as $\Delta G_s^0$, and for nonspecific interactions as $\Delta G_n^0$, then the total binding energy is the *sum* of $\Delta G_s^0$ and $\Delta G_n^0$. But the effective dissociation constant is equal to the *product* of $K_{ds}$ and $K_{dn}$, the constants for the specific and nonspecific interactions. A very weak nonspecific interaction in addition to the biospecific one can tip the balance between adsorption and nonadsorption.

This theory describing an affinity constant is rather simplistic, because in fact any adsorbent will have a range of affinity constants according to the accessibility of the individual ligands. There will be some tight-binding sites which are occupied first, and weaker ones will be occupied as more sample is applied. There may be some bleeding of protein from the weakest sites as the column is washed. A further complication is the possibility of multi-point attachment, in which an enzyme made up of to two or more subunits, each with a binding site, may be held by two or more ligands. Such binding would be very strong, only likely at high matrix ligand concentrations ($> 20$ μmol $mL^{-1}$). All such theoretical treatments assume that equilibrium conditions exist: this is only an approximation at the flow rates that are commonly employed, and some diffusional processes are so slow that the equilibrium assumption is far from valid.

In order to elute the protein from the column, the binding must be weakened. In most cases, a decrease of $\Delta G_s^0$ is sufficient, and this can be done by either nonspecific buffer changes such as increased salt concentration, or by specific methods (see below).

## Types of Ligand

In theory, any molecule can be used as a ligand in affinity chromatography, from small ones like

amino acids to large proteins and even supramolecular fragments such as membranes or whole cells. It must be possible to immobilize the ligand in such a way that it is still recognized by its biological partner. Thus, a random attachment of an antibody through lysines will allow only a small proportion of the antibody molecules to be oriented in an appropriate way to bind with their antigen, but a more directed attachment, for instance through the carbohydrate moiety on the Fc fragment, can increase this proportion significantly. Attachment of a ligand through an amine that is part of the biological recognition site will not produce a satisfactory affinity adsorbent, and some subtle chemistry may be needed to synthesize a ligand derivative that attaches elsewhere.

Highly specific adsorbents which are expected to interact with only one protein are the ideal, but may need to be synthesized individually (antibodies are a good example of this). On the other hand, group ligands which are expected to interact with a range of different proteins will obviously be less specific, but can be used for a wide range of different purifications. Not surprisingly, it is the latter which tend to be available commercially. An example is AMP-agarose, which interacts with enzymes that have ATP or $NAD^+$ as substrates. Despite the fact that AMP itself binds very weakly to most such enzymes, the additional nonspecific hydrophobic interactions with the hexyl spacer arm create sufficient binding energy. Lectins, such as concanavalin A which specifically binds to mannose residues in glycoproteins, have been extensively employed. Pseudo-affinity group adsorbents such as dye ligands have many advantages, including simplicity of synthesis.

Possibly the best known types of affinity adsorbents are antibodies, for purification of antigens; protein A and protein G for purification of antibodies; and an increasing range of affinity and pseudo-affinity materials for purification of recombinant fusion proteins. This last group will be discussed below, and antibodies (immunoaffinity chromatography) are described elsewhere.

## The Matrix

The vast majority of affinity chromatography adsorbents have made use of agarose as the base material, or matrix. Beads of diameters between 40 and 150 μm have been the most popular, and the agarose cross-linked to provide increased rigidity and temperature stability. But there are several propriety matrices, mostly synthetic materials, that are available as alternatives. These have mainly been developed as high resolution, high performance adsorbents as ion exchangers, and have the desirable properties such as fast flow rates, uniform bead diameters and rigidity under high pressure conditions. These are properties that are not often relevant to affinity methods, and at least on a large scale the extra cost of these materials compared with agarose may not be justified. It is always important that there should be a minimum of adsorption of proteins on the matrix itself, and carbohydrates such as agarose satisfy that requirement better than most other materials. Although we usually talk about affinity columns, there are several other ways of using an affinity adsorbent apart from in a column. Batchwise processing can be very successful if the affinity interaction is sufficiently strong, and other configurations include stacks of membranes as matrix, with the sample being forced through the membranes under pressure.

## The Elution Step

### General Elution

Having bound the desired protein to the affinity column, it must now be eluted. The standard way with simple ligands is to increase the salt concentration, or use a radical shift in buffer pH. This normally interferes with natural bonding between protein and ligand, thereby weakening the affinity constant between the two. But there are many cases in which increasing salt concentration is not appropriate, especially with antibody–antigen interactions. If the adsorption has a high hydrophobic contribution, then increasing salt concentration may increase the strength of binding. In that case elution may be achievable by a very low ionic strength buffer, in combination with a slightly alkaline pH. Nonionic detergents may also assist. With immunoadsorbents the elution is carried out by partially denaturing the antibody at low (2) or high (10) pH, or by chaotropic (structure-destabilizing) agents such as guanidine hydrochloride or sodium thiocyanate. The antigen is released, but if it is a protein, it might be denatured under these conditions.

Complete elution of all the protein from the column is fine if the adsorption has been specific, and only the desired component has been bound. But if unwanted proteins are bound in addition to the target component, then a more selective method is appropriate. The buffer conditions are adjusted so that the desired component is not quite eluted, but other proteins are, giving a preliminary clean-up. Then the conditions are adjusted again so that the desired component is just eluted, but other proteins remain on the

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column. Sometimes this can be useful, but it is far preferable to use affinity techniques at the elution stage as well as at the adsorption stage.

### Affinity Elution

Although it is hoped that only the desired component will bind to an affinity column, in many cases this is not so. For example, when a group adsorbent such as a nucleotide is used, there are likely to be many different enzymes with an affinity for that nucleotide which will bind to the column, and pseudo-affinity adsorbents such as dyes bind many proteins nonspecifically. Because of this, use of an affinity procedure during the elution can be highly beneficial. The principle is simple: free ligand is included in the elution buffer, and displaces the adsorbed protein from the immobilized ligand (**Figure 4**). The technique is called affinity elution, or biospecific elution. The displacement may occur at different pHs, different ligand concentrations, or salt concentration for each specifically bound protein that is on the column, since each is likely to have a different affinity constant. Thus, it is possible to elute the desired protein under conditions when few, if any, of the others accompany it. By applying affinity concepts at both the adsorption and the elution stages, a high degree of purification is obtained. The concentration of ligand used in the elution buffer must be sufficient to compete with the immobilized concentration, and generally at least 10 times the natural dissociation constant should be used. This can be quite costly on a large scale with some ligands.

Affinity elution is also valuable when the adsorption stage has been even less specific. The general concept of adding the ligand to the elution buffer so as to cause the desired protein to come off does not in itself dictate how the protein was bound in the first place. In fact, affinity elution has been very successful even with ion exchange adsorbents. The best examples are with cation exchangers, when binding of a negatively charged ligand decreases the strength of binding of the positively charged protein to the adsorbent. But the most generally used adsorbents in which affinity elution is applied are the pseudo-affinity dye adsorbents.

## Affinity Chromatography of Recombinant Proteins

During the last few years the use of affinity chromatography has become very widespread due to the ability, using molecular biology techniques, to modify proteins so that they can bind to specific adsorbents. The basic principle is illustrated in **Figure 5**. The gene encoding the protein is fused to DNA which encodes either a complete protein or a polypeptide that is to be used in the affinity process. The expressed protein is then readily purified from the host proteins by passing through the appropriate affinity adsorbent. Since only one adsorbent is needed for each particular system, one laboratory may use the same adsorbent for all its protein purifications. A brief list of some of the combinations of fusion/adsorbent is given in **Table 2**. These are all commercial products, and there is considerable competition, with new ones being introduced all the time. A popular term for the system is affinity tagging, with the tag being the fusion part. No one system is ideal for all proteins. In particular, the level of expression obtained can be dependent on the fusion type, and the maximum possible expression is required to optimize the overall process.

The end-product of the purification is not the original protein, but a fusion with the added protein or polypeptide. For many purposes this product is good enough, but there are methods of removing the fusion portion, generally by proteolysis. Vectors for creating fusion proteins include an amino acid sequence that is

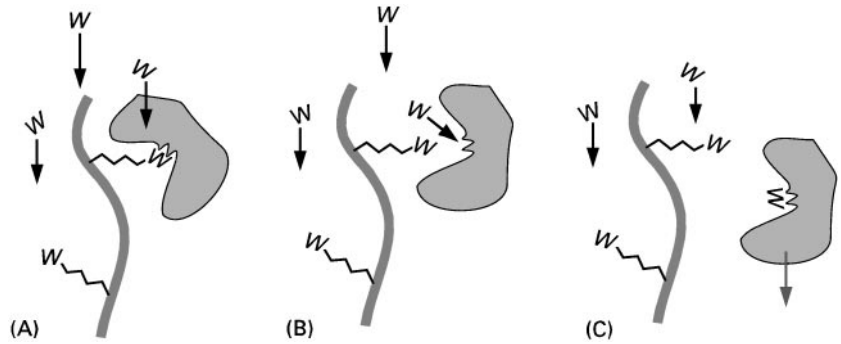


**Figure 4** Principles of affinity elution. The specifically bound protein is displaced by binding preferentially with the free ligand, *W*, present in the elution buffer. Other proteins which may be adsorbed nonspecifically do not interact with ligand *W*, and so remain on the column.

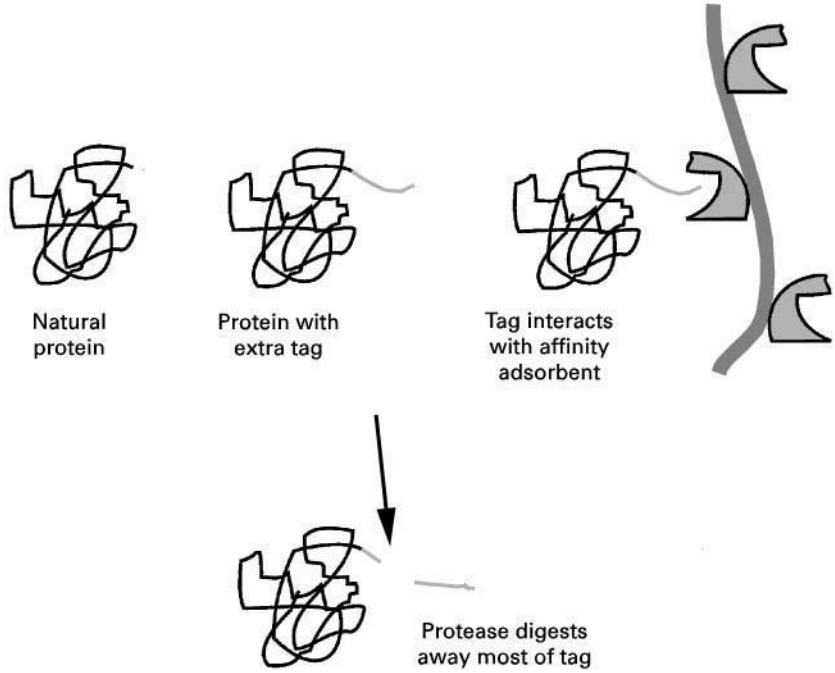


**Figure 5** Principle of affinity tagging. The protein is expressed in recombinant form with an extra polypeptide tag. This may be a few amino acids, or may be a complete protein. The affinity adsorbent recognizes the tag, whereas untagged proteins are not adsorbed. The tag may be removed with a protease, either while still on the column, or after elution.

recognized by a highly selective proteolytic enzyme such as a blood-clotting factor. Treatment with this enzyme, either before or after the fusion protein has been eluted from the affinity column, releases the original protein, though still with a few extra amino acids in most cases.

## Future Developments

We may assume that current trends will continue. As the number of gene sequences continues to expand, with the completion of the human genome project not far off, not to mention the many other genomes being sequenced, there will be more demand to express and purify these gene products. In many cases the actual nature of the protein will be unknown, with no assay available. By having a reliable fusion system, as outlined above, gene products can be isolated without knowing what their biological function is. So the use of standard affinity materials will be a major item in protein purifications in the near future. But there will still be a need for the more personal affinity system, for studying and isolating components of protein–protein or protein–DNA interactions. For this, the researcher needs a reliable activated matrix (such as those that have been commercially available for many years), to which to add the protein or DNA and get instant attachment.

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**Table 2** A selection of affinity tagging systems available commercially. Most tags are placed at the N-terminus of the expressing protein, but some, notably the hexahistidine, can be placed at either end

| *System*: *fusion protein* | *Affinity adsorbent* | *Size of fusion (kDa)* |
|---|---|---|
| Hexahistidine | Immobilized metal: Ni or Co | 1–2 |
| Glutathione *S*-transferase | Glutathione | 26 |
| Maltose-binding protein | Amylose | 38 |
| Cellulose-binding domain | Cellulose | 35 |
| T7 Polymerase (peptide) | Monoclonal antibody | 1 |
| Protein A (partial) | IgG | 14 |
| Biotinylation site | Streptavidin | 1 |
| Various epitopes | Monoclonal antibodies | 1 + |

*See also:* **I/Affinity Separation. II/Affinity Separation:** Affinity Membranes; Affinity Partitioning in Aqueous Two-Phase Systems; Aqueous Two-Phase Systems; Biochemical Engineering Aspects; Covalent Chromatography; Dye Ligands; Hydrophobic Interaction Chromatography; Immobilized Boronates and Lectins; Immobilized Metal Ion Chromatography; Immunoaffinity Chromatography; Imprint Polymers; Rational Design, Synthesis and Evaluation: Affinity Ligands. **Appendix 1/Essential Guides for Isolation/Purification of Enzymes and Proteins. Essential Guides for Isolation/ Purification of Immunoglobulins. Appendix 2/Essential Guides to Method Development in Affinity Chromatograhy.**

## Further Reading

Coligan J, Dunn B, Ploegh H *et al.* (eds) (1995) *Current Protocols in Protein Science*. New York: John Wiley.

Harris ELV and Angal S (eds) (1990) *Protein Purification Applications*; *A Practical Approach*. Oxford: IRL Press.

Hermanson GT, Mallia AK and Smith PK (1992) *Immobilized Affinity Ligand Techniques*. New York: Academic Press.

Kenny A and Fowell S (eds) (1992) *Practical Protein Chromatography*. New Jersey: Humana Press.

Matejtschuk P (ed.) (1997) *Affinity Separations*: *A Practical Approach*. Oxford: IRL Press.

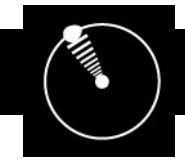
Ostrove S (1990) Affinity Chromatography. *Methods in Enzymology* 182: 357–379.

Scopes RK (1993) *Protein Purification*, *Principles and Practice*, 3rd edn. New York: Springer-Verlag.

Scopes RK (1997) Protein purification in the nineties. *Biotechnology and Applied Biochemistry* 23: 197–204.

Turkova J (1993) *Bioaffinity Chromatography*. Journal of Chromatography Library, vol. 55. Amsterdam: Elsevier.

# CENTRIFUGATION



## Analytical Ultracentrifugation

**J. L. Cole**, Merck Research Laboratories, West Point, PA, USA

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Analytical ultracentrifugation (AUC) involves the measurement of the radial concentration gradients of molecules created by the application of centrifugal force. In contrast to preparative centrifugation, which is used to fractionate mixtures, AUC is a purely analytical technique. Since the pioneering work of Svedberg and associates in the 1920s, AUC has been employed to characterize the mass, size, shape and association properties of macromolecules in solution. The technique has been broadly applied to research problems in biochemistry, molecular biology and polymer sciences and has also found practical applications in the pharmaceutical and biotechnology industries. Some of the most attractive features of AUC are:

1. Versatility: a wide variety of samples can be examined by AUC, including molecules ranging in size from sucrose to virus particles.
2. Rigor: AUC experiments are directly interpreted in the context of thermodynamic and hydrodynamic theory, so it is not necessary to run standards to calibrate each experiment. Also, because the experiments are performed in free solution there are no complications due to interactions with matrices or surfaces that can complicate interpretation of other types of measurements.
3. Convenience: recently, new instrumentation (Beckman Coulter XL-A and XL-I) and data analysis methods have made AUC much more convenient and accessible to the general biochemistry and polymer science communities. In contrast to earlier instruments, experiments are easy to set up and centrifugation parameters and data acquisition are all under computer control. In addition, powerful desktop computers and new software have greatly accelerated the data analysis process and have also extended the capabilities of AUC.

A complete treatment of the theory and applications of AUC is beyond the scope of this article, and the interested reader is referred to the Further Reading section.

## Theoretical Background

The analytical ultracentrifuge is used to perform two different types of experiments, referred to as sedimentation velocity and sedimentation equilibrium. Sedimentation velocity is a hydrodynamic technique and is sensitive to both the mass and shape of a macromolecule. It can be used qualitatively to